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Exploration of the Selectivity and Retention Behavior of Alternative Polyacrylamides in Temperature Responsive Liquid Chromatography

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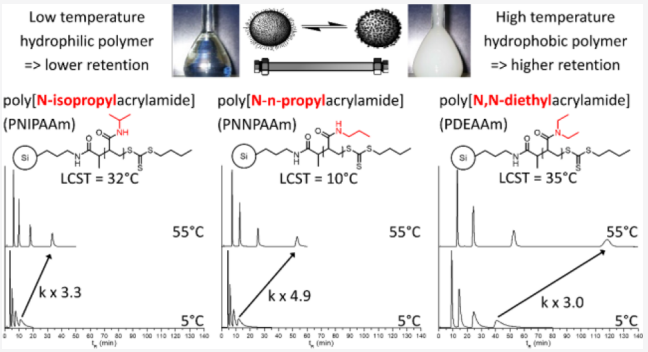
ABSTRACT: Temperature responsive liquid chromatography (TRLC) allows for separation of organic solutes in purely aqueous mobile phases whereby retention is controlled through temperature. The vast majority of the work has thus far been performed on poly[*N*-isopropylacrylamide] (PNIPAAm)-based columns, while the performance of other temperature responsive polymers has rarely been compared under identical conditions. Therefore, in this work, two novel TRLC phases based on poly[*N*-*n*-propylacrylamide] (PNNPAAm) and poly[*N*,*N*-diethylacrylamide] (PDEAAm) are reported and compared to the state of the art PNIPAAm based column. Optimal comparison is thereby obtained by the use of controlled radical polymerizations, identical molecular weights, and by maximizing carbon loads on the silica supporting material. Analysis of identical test mixtures of homologue series and pharmaceutical samples revealed that PNNPAAm performs in a similar way as PNIPAAm while offering enhanced retention and a shift of the useable temperature range toward lower temperatures. PDEAAm offers a range of novel possibilities as it depicts a different selectivity, allowing for enhanced resolution in TRLC in, for example, coupled column systems. Reduced plate heights of 3 could be obtained on the homemade columns, offering the promise for reasonable column efficiencies in TRLC despite the use of bulky polymers as stationary phases in HPLC.



Temperature responsive liquid chromatography (TRLC) is a promising alternative separation technique allowing for reversed phase type separations under *purely* aqueous separations, whereby retention is controlled through column temperature as compared to the organic modifier content in conventional RPLC.[1−3] This peculiar retention behavior is achieved by employing a particular class of smart (or stimuli-responsive) polymers that are able to change their water solubility as a function of temperature, whereby once a certain temperature is exceeded they are no longer water-soluble. The specific temperature at which this transition occurs depends on the type and molecular weight of the polymer used, and is called the lower critical solution temperature (LCST).[4] In TRLC, these polymer chains are most commonly covalently anchored to silica particles and packed into stainless steel HPLC columns.[1,2,5] Alternatively, also the use of temperature responsive polymer monoliths is being explored.[6]

The utilization of these stationary phases in LC allows obtaining increased or reduced retention at higher or lower temperatures, respectively. The former is caused by the formation of an apolar layer on the surface of the stationary phase owing to the dissolution (demixing) of the polymer from the aqueous mobile phase at higher temperatures. Correspondingly, cooling down the column resolubilizes the polymer chains eliminating the retentive layer. This phenomenon is not an on/off type event, but a gradual change in retention around the LCST due to the dispersity of macromolecules, whereby each polymer length depicts a slightly varying dissolution temperature. The exact nature of the retention behavior and selectivity in TRLC is currently not fully understood, but previous research has established that this is mainly RPLC like, offering increased retention with increasing hydrophobicity.[7] Additional contributions, reminiscent of the interactions with polar embedded or mixed mode type of RPLC phases, have also been observed causing significant retention increases or selectivity changes for analytes comprising polar groups or unsaturated bonds. It appears that this is most likely due to both the polarity inducing effect and hydrogen bond capabilities of the amide bond in the polymers.[7−9] The principles of this purely aqueous separation mode have been demonstrated through various examples including, for example,

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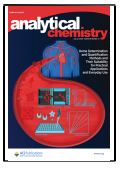

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the separation of pesticides, pharmaceuticals, steroids, or of some peptides or proteins.[2,10−13] More recently, promising refocusing and modulation benefits have been demonstrated for the combination of TRLC and RPLC in heart-cutting and comprehensive 2D-LC, respectively.[7,14]

Strikingly since its conceptualization in the nineties, most of the work has been focused on the development of columns based on poly(*N*-isopropylacrylamide) (PNIPAAm) in homo- or copolymers.[15,16] This is ascribed to the fact that the LCST (32 °C) of this particular well-known polymer lies within a suitable temperature range for HPLC, but also because this is close to the physiological temperature, which has led to a vast amount of earlier research on this biocompatible polymer in unrelated fields (such as drug delivery and tissue engineering devices).[17] Despite successful examples described above based on PNIPAAm columns, the development of HPLC methods centered solely on this polymer has proven challenging due to a variety of reasons. These include among others: the sometimes limited peak capacities, the potentially problematic retention for polar or ionic solutes, and the change in retention as a function of temperature which has often been lower than adequate for broad-scale implementation of TRLC.[5,18]

One of the investigated routes to overcome the limited retention of pure PNIPAAm-based columns has been based on copolymerization with more hydrophobic monomers.[19] The copolymerization of NIPAAm with hydrophobic monomers, such as *n*-butyl methacrylate or *n-tert*-butylacrylamide, could increase the hydrophobicity of the formed polymer with an accompanying drop of the LCST, allowing for enhanced retention. However, a disadvantage of this approach is that the fraction of these additional monomers needs to be kept relatively low (compared to NIPAAm) in order to keep the polymer water-soluble, while the lowered LCST results in a higher retention at the lowest temperature due to a higher inherent apolarity of the formed polymer.[1,20−22] Alternatively, copolymers with pH-sensitive groups, such as a weak acid (carboxylic acid) or a weak base (tertiary amine) have also been studied allowing obtaining mixed-mode columns whereby retention is also based on electrostatic and hydrophobic interactions. Versatile phases are thereby obtained as the LCST of such polymers becomes pH tunable due to polarity variations caused by the protonation/deprotonation of the pH sensitive group.[23−30] Similarly, permanently charged anionic/cationic monomers have also been introduced in the copolymers.[31,32] Recently, aromatic monomers have been copolymerized with NIPAAm with the aim of introducing $\pi-\pi$ stacking interactions.[18,33−35]

Although copolymers can allow for enhanced retention of, for example, fairly dedicated biomolecules through combined retentive effects, the approach stands somewhat in contrast with the development of a more generic form of TRLC. This because it does not simultaneously allow for both a large flexibility in retention modulation (as the polarity change is decreased due to the addition of the apolar monomer) while offering the promise for faster mass transfer typically found in the fast on/off kinetics of hydrophobic interactions in RPLC. It can also be hard to understand the origin of retention when mixed-mode mechanisms are involved.

The technique of copolymerization is also prone to the occurrence of slight variations in monomer ratios leading to enhanced column-to-column variations in LCST. In contrast to this, the study of alternative homopolymers to PNIPAAm might allow for more broadly applicable TRLC columns. In this case, the LCST and the retention behavior is only determined by the polymer length, the dispersity, the carbon load on the silica and the monomer used. In principle a broad number of monomers could be suitable for TRLC, as all water-soluble polymers depict an LCST.[4] Although the latter will arguably often occur in an unusable temperature range, the work performed on alternative homopolymers in TRLC has been rather scarce. In this way, for example, poly(acryloyl-L-proline methyl ester) (LCST = 21 °C) has been assessed and compared to PNIPAAm based TRLC, allowing for alternative novel selectivity tailored toward derivatized amino acids while preserving temperature responsive activity.[36] Similarly, poly[*N*-vinylcaprolactam] (PVCL) has also been tested as homopolymer in TRLC, illustrating some tolerance toward the use of ethanol as cosolvent with water.[5] Also some non-PNIPAAm based copolymers have been tested, including an ethylene glycol and an oxazoline based copolymer.[19,37,38]

Because of the overall obtained subpar performance of these new columns, compared to PNIPAAm based TRLC, and because rigorous comparison between novel homopolymers and the former has been missing, in this work alternative more retentive *N*-alkylamide-based polymers are introduced for TRLC and systematically compared with PNIPAAm under comparable conditions (Figure 1). This includes poly[*N*-*n*-propylacrylamide] (PNNPAAm), which is a structural isomer of the commonly used PNIPAAm depicting a much lower LCST of 10 °C, allowing for investigation of the effects of the LCST on the resulting retentive properties.[39] The second one is poly[*N*,*N*-diethylacrylamide] (PDEAAm), comprising a comparable LCST (35 °C) to PNIPAAm, while offering insight into the contribution of the amide hydrogen to the TRLC properties.[40]

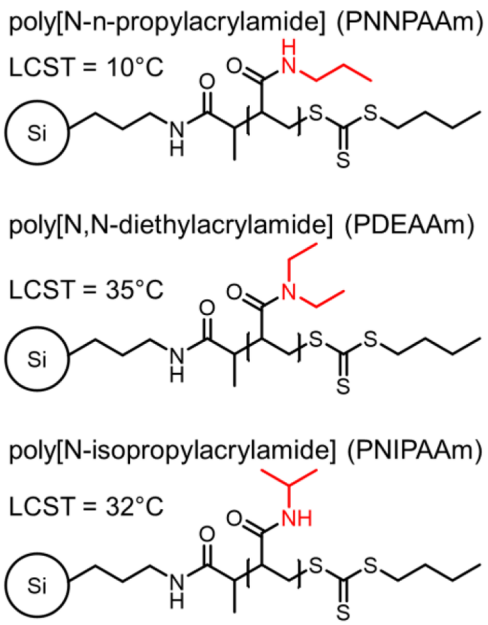

Figure 1. Structural representation of the three synthesized temperature responsive stationary phases with their respective LCST's.

EXPERIMENTAL SECTION

Chemicals and Reagents. HPLC grade acetonitrile (ACN), acetone, methanol (MeOH) and dichloromethane (DCM), were obtained from Sigma−Aldrich (Steinheim,

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Germany). Milli-Q grade water (18.2 mΩ) was purified and deionized in-house by a Milli-Q plus instrument from Millipore (MA). Formic acid (FA) was supplied by Acros (Geel, Belgium). *N*-isopropylacrylamide (NIPAAm) and diethylacrylamide were obtained from TCI EUROPE N.V. (Zwijndrecht, Belgium). *N,N'*-dicyclohexylcarbodiimide (DCC), *N*-hydroxysuccinimide (NHS) and 2,2-azobis-(isobutyronitrile) (AIBN) were also from Sigma-Aldrich. NIPAAm and AIBN were recrystallized twice in hexane and methanol, respectively, and subsequently dried in vacuo prior to use. As RAFT agent 2-{[(butylsulfanyl)- carbonothioyl]-sulfanyl}-propanoic acid was synthesized following a procedure from literature.[41] *N*-propylacrylamide (NNPAAm) was also synthesized according to a procedure from literature.[42] Diethyl ether (DEE) and petroleum ether 40−60 °C were obtained from Honeywell (Morris Plains, NJ) and chem-lab (Zedelgem, Belgium), respectively. Dry DMF was obtained from a custom-made JW Meyer solvent purification system and was dried over aluminum oxide columns. 100 × 4.6 mm ID stainless-steel columns were obtained from Idex (Lake Forest, IL, type isobar). 3-Aminopropyl-functionalized silica particles with an average particle size of 5 μm and pore size of 100 Å were purchased from Fuji Silysia Chemical (Kasugai, Japan).

The test mixture for evaluating the temperature responsive behavior of each polymer, consisted of compounds with varying functional groups. Methoxy-, ethoxy-, butoxybenzene as well as propyl and butyl benzoate originated from Acros; while methyl-, ethyl-, propyl-, and butylparaben together with propriophenone, acetophenone, and benzophenone were from Sigma-Aldrich. *n*-hexanophenone and *n*-butyrophenone were purchased from Janssen Chimica (Beerse, Belgium). Stock solutions of 1 or 2 mg/mL were prepared in ACN, according to the solubilities of the components. A mixture of all components was then prepared in H_2O/ACN (60:40) in concentration of 100 μg/mL.

The steroid mixture comprised methylprednisolone, prednisolone, cortexolone, hydrocortisone, hydrocortisone 21-acetate, cortisone 21-acetate, fluoxy-mesterone, beta-methasone 21-acetate, testosterone, and methyltestosterone, all obtained from Sigma−Aldrich as well as triamcinolone acetonide, supplied by Steraloids (Newport, RI). The barbiturates mixture comprised amobarbital, hexobarbital, secobarbital, butalbital, phenobital, pentobarbital from Sigma−Aldrich. For both samples stock solutions of 2 or 1 mg/mL were prepared in ACN or H_2O/ACN (50:50) respectively, according to the solubilities of the components. The sample for analysis was prepared with a concentration of 100 μg/mL in H_2O/ACN (60:40).

Column Synthesis. All polymers were synthesized according to the following procedure:

Polymer synthesis: The linear polymer was synthesized through reversible addition−fragmentation chain-transfer (RAFT) polymerization. Monomer (0.1 mol), AIBN (0.2 mmol) and 2-{[(butylsulfanyl)- carbonothioyl]sulfanyl}-propanoic acid (2 mmol) were dissolved in dry DMF. The concentration of monomer was fixed at 2 M. After purging with nitrogen for 20 min, the flask was immersed in a preheated oil bath of 65 °C. The conversion of monomer and molecular weight of polymer were monitored by GC and SEC (cf. Supporting Information). The polymerization was terminated by cooling the flask in liquid nitrogen after monomer conversion has exceeded 90%.

Polymer Activation. The terminal carboxylic end group was activated with *N*-hydroxysuccinimide. The polymer mixture obtained during the polymer synthesis was cooled to 0 °C. NHS (20 mmol) was added to the mixture and stirred until dissolved. DCC (20 mmol) was dissolved in 10 mL dry DMF and added dropwise to the polymer reaction solution under vigorous stirring. The reaction was left to stir in an ice bath for 2 h and stirring was continued at room temperature for 12 h. The solvent was evaporated under reduced pressure and purification was done by precipitation of the polymer from THF in DEE (PNIPAAm and PNNPAAm) or petroleum ether (PDEAAm).

Coupling Reaction. The polymer (5 g) and silica (5 g) and dry DMF (40 mL) were sonicated for 2 h and gently shaken at room temperature for 120 h, keeping the silica in suspension. Purification was done by repetitively ultrasonicating the silica in acetone and filtering over a glass filter. TGA analysis was performed to determine the attached polymer through pyrolysis of the polymer.

Column Packing. 100 × 4.6 mm ID stainless-steel columns were slurry-packed with H_2O/ACN 30/70 by a Haskel air-driven pump (Burbank, CA). For the slurry, 2.5 g derivatized silica was suspended in 20 mL H_2O/MeOH 50/50. After packing, the columns were conditioned with water and ACN until a stable UV-signal was obtained.

Instrumentation and Data Analysis. Cloud points were measured on a Crystal16 parallel crystallizer turbidimeter developed by Avantium Technologies connected to a recirculation chiller and dry compressed air. Aqueous polymer solutions (5 mg/mL) were heated from 3 to 45 °C with a heating rate of 1.0 °C/min followed by cooling to 3 °C at a cooling rate of 1.0 °C/min. This cycle was repeated three times and the cloud point temperatures (T_{CP}'s) were defined as the temperature at 50% transmittance in the second heating run. These conditions were based on a recent literature procedure, which supports the selection of a slightly faster heating and cooling rate.[43] Even though the latter could introduce a slight measurement error, the inherent error margin involved when determining T_{cp}, combined with the inability of this data to mimic the behavior inside the column, means that this data can merely serve as an indication of the T_{cp} differences between the polymers used.[43] This thus did not warrant the use of significantly longer analysis times.

Size exclusion chromatography (SEC) measurements were done on an Agilent 1260-series HPLC system (Agilent, Waldbronn, Germany) with two PLgel 5 μm mixed-D columns (300 × 7.5 mm) and a mixed-D guard column(50 × 7.5 mm) (Agilent) in series. Detection was performed using a 1260 diode array detector, a refractive index detector (RID) and a multiangle light scattering (MALS) detector (WYATT technology, Santa Barbara, CA). Dimethylacetamide containing 50 mM lithium chloride was used as eluent at an optimized flow rate of 0.5 mL/min.[44] The setup is calibrated against poly(methyl methacrylate) (PMMA) standards from Polymer Standards Service (Mainz, Germany). The SEC-spectra were analyzed using the Agilent Chemstation software with the GPC add on and the LS results were analyzed with the provided Astra 7 software designed by Wyatt Technology.

Thermogravimetric analyses were performed on a Mettler-Toledo TGA/SDTA851e instrument (Mettler-toledo, Greifensee, Switzerland) under an oxygen-rich atmosphere at a heating rate of 10 °C/min from 25 to 800 °C. The

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thermograms were analyzed using STARe software from Mettler-Toledo.

The TRLC measurements were performed using an 1100 series HPLC system (Agilent Technologies, Waldbronn, Germany). The system was constructed out of an 1100 binary pump equipped with an 1100 degasser, an 1100 auto injector and an 1100 variable wavelength detector (VWD) equipped with micro flow cell. The column temperature was controlled using a water/glycol bath (Julabo, Seelbach, Germany, model F10).

All chromatograms were generated using OriginPro 8.5 (OriginLab Corporation, Northampton, MA.).

Chromatographic Conditions. As mobile phase 0.1 vol % aqueous FA was used at flow rates between 125 and 2500 μL/min. The column temperature was controlled at either 5, 15, 25, 35, 45, 55, or 65 °C. The injected volume was 5 μL and the separation was monitored by the VWD detector at a wavelength of 254 nm, except for the barbiturates sample which was measured at 210 nm.

All reversed phased measurements were performed on a 50 × 4.6 mm, 3.5 μm XBridge C18 column (Waters, Milford, MA.) at room temperature.

RESULTS AND DISCUSSION

Synthesis of Acrylamide Based Stationary Phases. In order to allow deeper understanding of the phenomena involved in TRLC and to envisage broader implementation of this chromatographic approach, the use of controlled polymerization strategies is arguably essential. Although free radical polymerization strategies have successfully been reported in TRLC, the larger polymer and stationary phase variability involved can entail retention time reproducibility concerns. The temperature at which the polymers precipitate from solution is concentration dependent and is called T_{CP}. The commonly reported polymer dependent LCST-value is derived from these T_{CP} values, as it represents the lowest T_{CP} obtained when measuring the full range of polymer concentrations.[4] As the T_{CP} is not only dependent on the polymer concentration, but also on a host of structural parameters (such as length and end group structure) of the polymer, enhanced control of the polymerization process also eventually allows for improved steering of the retention and gradient times in TRLC such as to obtain optimized separations.[45] RAFT polymerization is employed in this work, which is a reversible-deactivation radical polymerization technique leading to well-defined polymer with narrow molecular weight distribution.[18]

To be ideally utilizable as stationary phase in TRLC, the polymer chains should be of modest, yet not too short, lengths in order to exhibit suitable temperature responsive behavior while avoiding slowing down mass transfer phenomena in the stationary phase due to excessive polymer molecular weights. Last but not least, the availability of polymers with the same molecular weight and distribution was also considered essential in this study such as to allow for the least biased comparison of the influence of each polymer in TRLC (Table 1). To be able to unequivocally corroborate all results, the entire polymer synthesis and coupling chemistry processes, together with the column packing and physical and chromatographic measurements, were also repeated for each type of column described. The duplicate data is provided in the Supporting Information. Milford) at room temperature.

Table 1. Properties of the Synthesized Polymers[a]

	T_{CP} (°C)	Mn (kDa)	Đ	loading (%)
PNIPAAm	29.5	5.3	1.22	27.5
PDEAAm	28.0	5.3	1.13	22.25
PNNPAAm	19.5	5.9	1.10	25.25

[a]A more detailed analysis of the polymer properties can be found in the Supporting Information.

FIGURES OF MERIT OF THE DEVELOPED PHASES

In order to evaluate and compare the temperature responsive effect between the phases developed in this work and with literature, the retention of a sample comprising four parabens was measured at different temperatures spanning the LCST's of the respective polymers (Figure 2). As the retention of the PNIPAAm column corresponds well with earlier comparable columns (obtained by free radical polymerization), this column is a suitable reference point to allow comparison with the other phases.[2]

The Van't Hoff plots (Figure 3) and the corresponding chromatograms (Figure 2) show that all phases indeed depict temperature responsive behavior within a specific polymer-dependent range. From a chromatographic point of view, the temperatures above the LCST are the most useful as they allow obtaining the necessary retention for separation together with acceptable separation efficiencies. The latter as consequence of the occurrence of thin layer of precipitated polymer phase. The main practical purpose of the lower temperatures is to allow for enhanced eluotropic strengths when operating gradient analyses.

Above the temperature range during which retention increases due to the precipitating polymer, conventional chromatographic thermodynamic behavior takes again over, resulting in a loss in retention as a function of temperature. A good correspondence is observed between the temperatures at which these phenomena occur and the corresponding LCST's of the different polymers. The PNNPAAm-based column, which has the lowest LCST, shows the lowest temperature responsive range (<5−45 °C), whereas the PNIPAAm-based column, with the highest LCST, depicts the highest temperature responsive range (15−->60 °C). The PDEAAm-based column with an LCST, slightly below, but close to the one of PNIPAAm, shows a comparable range (10−55 °C). Interesting is the observation that in all cases increases in retention are observed for temperatures of 25−30 °C above the LCST, illustrating that the sharp on/off mechanisms observed in solution are more attenuated when immobilizing the polymers on solid supports. Note that such gradual conversion is beneficial for chromatography as it allows enhanced retention time optimization in HPLC. Additional observations could be made from Figure 3 with regards to the slope of the curve and the total change in retention between the different polymers, mainly between PDEAAm and both PNIPAAm and PNNIPAAm. While the nature of this behavior could be accredited to the differences in polymer structure, the most probable explanation would however be that this is caused by the differences in polymer loading.

The most relevant observation when comparing the retention on the three types of columns is the much higher retention obtained for the parabens for the PDEAAm column, and also to some extend for the PNNPAAm column as compared to the traditional PNIPAAm based columns. In order to both verify these observations and to further compare

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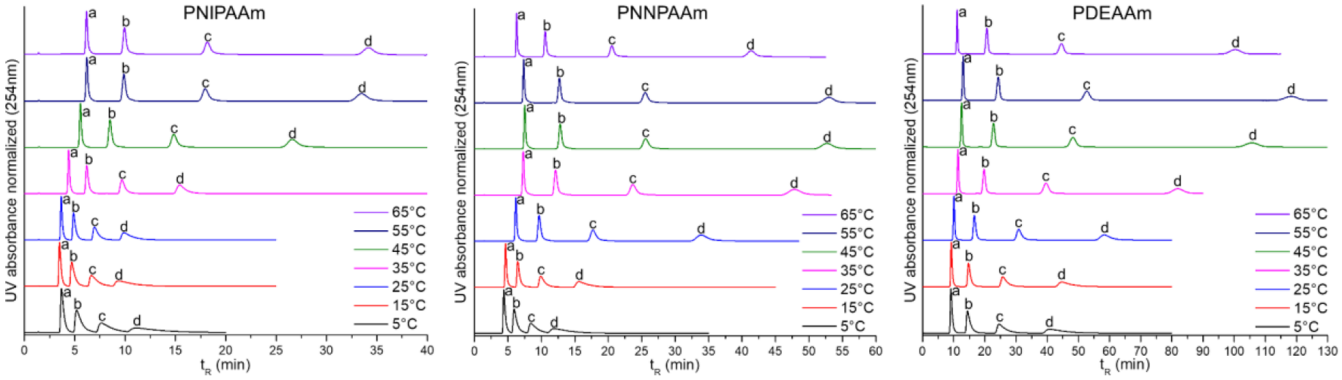

Figure 2. Temperature responsive effect of all synthesized columns for the separation of parabens in water (+0.1% formic acid) as mobile phase and flow rate of 1.0 mL/min. Compound labels: (a) methylparaben, (b) ethylparaben, (c) propylparaben, (d) butylparaben.

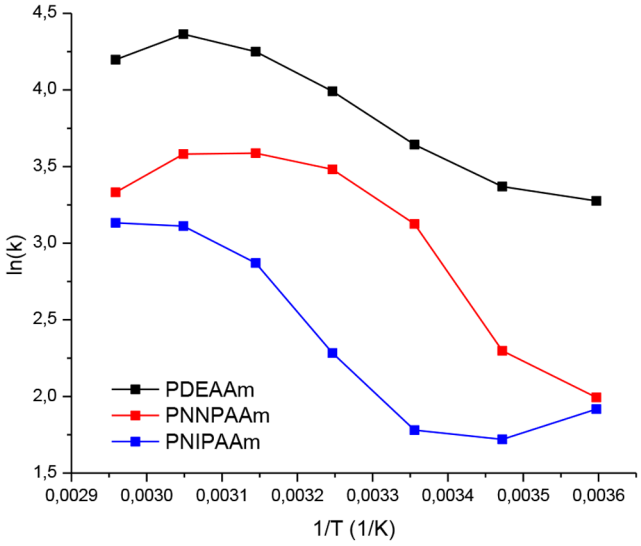

Figure 3. Van 't Hoff plots of butylparaben on all synthesized columns at 1.0 mL/min depicting the differences in retention and temperature responsive effect.

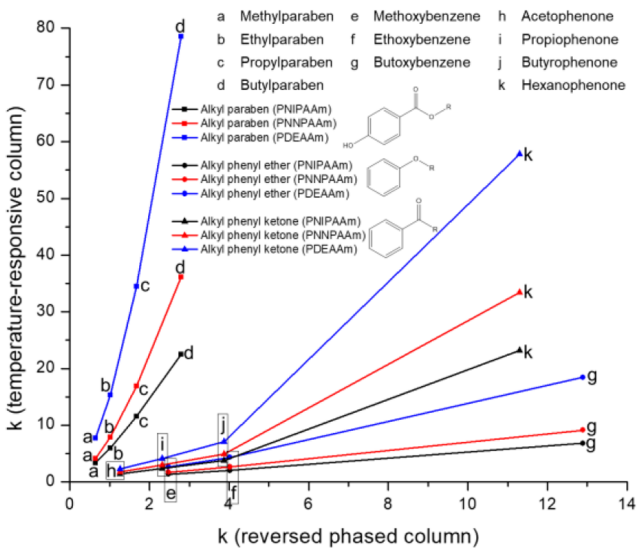

Figure 4. Representation of the correlation between the retention factors of the selected standard compounds on the synthesized temperature responsive columns and on a RP column. The retention factors on the TR column were measured at 55 °C using 1.0 mL/min of 0.1 vol % aqueous FA as mobile phase, and the *k* values on the RP column were measured at 1 mL/min using 0.1 vol % aqueous FA and 0.1 vol % FA in ACN at a 50/50 ratio.

the selectivity of the phases, a test mixture containing homologue series of three compound classes was subsequently separated on all columns and compared to the retention on a conventional (C18) reversed phase column (Figure 4). This graph depicts a comparable linear increase in retention for the temperature responsive and reversed phased columns for a homologue series of analytes; however, a significant increase in retention is present in all temperature responsive columns for solutes comprising also polar groups when compared to the reversed phased retention.[7] It also shows that the PNNPAAm based column depicts an in-between retention between the less and more retentive PNIPAAm and PDEAAm phases, respectively. The slightly higher retention of the PNNPAAm based column compared to the PNIPAAm based column, can easily be appropriated to the slight increase in hydrophobicity between the columns due to the more apolar *n*-propyl side chain compared to the smaller isopropyl isomer chain. This also explains the lower LCST of PNNPAAm, as a consequence of the decreased solubility of the side chain. Note also that significantly higher PNNPAAm retention is obtained despite a lower carbon load (Table 1).

However, the large increase in retention obtained for the PDEAAm column cannot be explained based on LCST, as it depicts a comparable conversion temperature compared to PNIPAAm. Although the exact nature of the retention and selectivity behavior in TRLC still remain hypothetical, a reasonable assumption of the impact of the structural differences can be made with regards to the identified selectivity and retention contributions in TRLC. Although its initial solubility might not differ that much from PNIPAAm, the removal of the amide hydrogen and the introduction of a bulkier side chain in PDEAAm could, after precipitation, cause a more hydrophobic surface. This due to the fact that both the absence of hydrogen bond donating capabilities could make the surface less appealing for polar compounds and the bulkier side chains could provide better shielding. Additionally, these changes would undoubtedly also have a significant impact on the observed mixed-mode/polar embedded RPLC like retention and selectivity contributions for polar groups and unsaturated bonds in TRLC.

Next to the retentive characteristics of the columns, the efficiency of the temperature responsive columns was also

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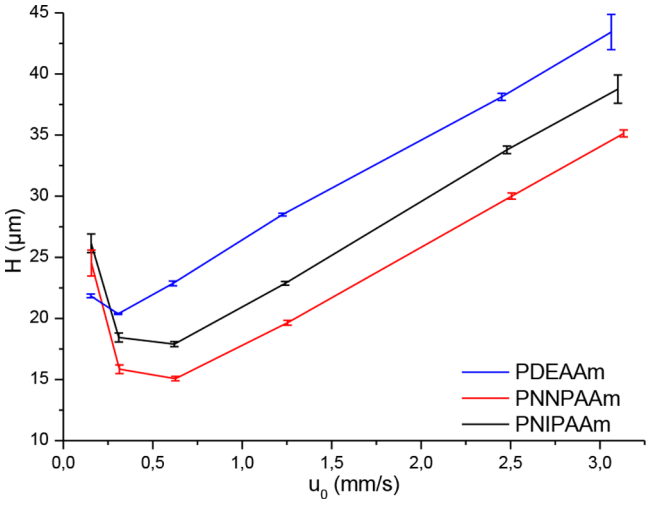

Figure 5. Van Deemter plot of the synthesized columns at 55 °C. The measurements were performed in triplicate and represent the averaged plate height of methylparaben and ethylparaben.

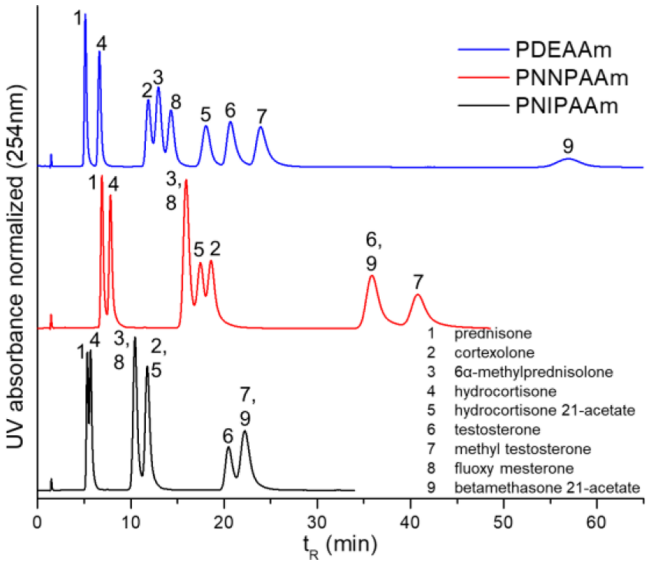

Figure 6. Analyses of the steroid sample mixture on all synthesized columns at 55 °C and at a flow rate of 1.0 mL/min.

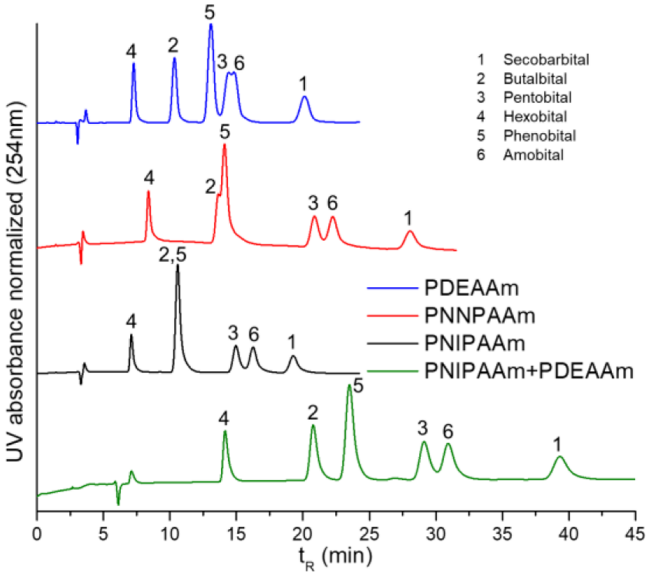

Figure 7. (top) Comparative analyses of a barbital sample on all column types at 55 °C and at a flow rate of 1.0 mL/min. (bottom) Resolved chromatogram by combining selectivities in a coupled column system (PNIPAAm + PDEAAm). Flow rate 1.0 mL/min.

evaluated as a function of the flow rate as represented in Figure 5. This graph reveals a roughly comparable performance for all in-house packed columns, with the best packed column (PNNPAAm in this particular case) reaching 15 μm in average plate height (reduced plate height = 3) for well retained solutes. Similar to prior observations also these RAFT based phases (comprising polymers with a narrow MW distribution) depict fairly steep C-term contributions requiring optimal operation at relatively low flow rates (about 3−4 times slower compared to conventional RPLC).[46] The inherent temperature dependency of this steep C-term also explains the origin of the peak broadening observed at lower temperature in Figure 2.[46] Even though this behavior is a characteristic of polymer-based stationary phases in general, it has been shown that, taking into account the typically higher operating temperatures, the use of polymer phases does not fundamentally hinder approaching the H ∼ 2dp limit observed for conventional (fully porous silica based) columns.[46] It is expected that this behavior could be significantly reduced through careful selection and extensive control of the polymer properties, as the required temperature responsive effect has already been observed for remarkably short polymer lengths.[45] Additionally, next to further optimizing the packing conditions, both the use of a reduced particle size or core−shell particles should further improve the A and C-term contributions in the observed plate heights.

Implementing the Different Stationary Phases for the Separation of Pharmaceutical Samples. In the framework of increasing complexity of pharmaceutical drugs, the availability of alternative separation selectivities such as purely aqueous TRLC can offer various promising possibilities in conventional 1D HPLC or in comprehensive or heart-cutting 2D-LC approaches.[7] The selectivity of the used phases was therefore also assessed with a mixture of steroids and barbiturates under retentive isothermal conditions (Figures 6 and 7). Although again enhanced retention was obtained for various steroids and barbitals on the PDEAAm and PNNPAAm columns, these chromatograms also reflect the occurrence of quite significant selectivity changes between the columns. The PNNPAAm based column reinforced the previous findings, as it showed a significant gain in retention and thus separation compared to the traditional PNIPAAm based column, while largely mirroring its selectivity. On the other hand, for the PDEAAm based column, although a slightly enhanced retention was observed compared to PNIPAAm, for most solutes a distinct selectivity difference was observed compared to both the PNIPAAm and PNNPAAm based phase. This deviating selectivity and retention behavior can, as previously explained in more detail, be attributed to the structural differences between the polymers.

This opens up the possibility of selectivity tuning by, for example, coupling of TRLC columns. This is illustrated for the analysis of a number of barbiturates, whereby no separation of overlapping solutes is obtained on the pure PNIPAAm and PDEAAm phases, but resolved peaks are obtained on a coupled column combining the selectivities (Figure 7).

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CONCLUSIONS

In this work, two novel temperature responsive stationary phases were introduced and compared to a current state-of-the-art PNIPAAm based column with the aim to broaden the understanding and possibilities of TRLC.

Next to an observed correlation between the polymer's LCST values, cloud points, and chromatographic retention, this study also showed the occurrence of significant and exploitable retention and selectivity differences occurring between the polymers.

These new types of stationary phases open new prospects for temperature responsive liquid chromatography. The PNNPAAm based column offers a lower temperature responsive range while still maintaining a high retention and exemplary temperature responsive effect. This could be exploited for the separation of, for example, temperature labile compounds. The PDEAAm based column, on the other hand, depicts an interesting alternative selectivity, offering novel prospects for the separation of poorly resolved or of insufficiently retained compounds. Concomitantly, this article also demonstrates that the RAFT synthesis pathway can be reliably used for the reproducible synthesis of high performance temperature responsive columns.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.analchem.0c01321.

Characterization of the temperature-responsive polymers; Additional and duplicated data of all the polymers and columns used (PDF)

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Notes

The authors declare no competing financial interest.

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